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ORIGINAL ARTICLE

Highly sensitive stripping voltammetric determination of a biomolecule, pyruvic acid in solubilized system and biological fluids

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Square-wave adsorptive stripping voltammetry;
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Abstract Electroreduction and adsorption behavior of pyruvic acid were studied in the presence of cetyltrimethylammonium bromide by using cyclic voltammetry, differential pulse adsorptive stripping voltammetry and square-wave adsorptive stripping voltammetry at HMDE. The reduction peak current increases in the presence of CTAB. These fully validated sensitive and reproducible adsorptive stripping voltammetric techniques were applied for the trace determination of the pyruvic acid in biological samples. Pyruvic acid shows a single irreversible reduction peak at −1.35 V in ammonia buffer of pH 8.2 ± 0.01. Different experimental conditions were examined by using differential pulse adsorptive stripping voltammetry and square wave adsorptive stripping voltammetry. These electroanalytical procedures enabled to determine pyruvic acid in the concentration range 0.004–0.036 mM for both DPAdSV and SWAdSV. The detection and quantification limits were found to be 6.12×10^{-6} and 2.0×10^{-5} mM for DPAdSV and 1.12×10^{-7} and 4.4×10^{-6} mM for SWAdSV, respectively.

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1. Introduction

Pyruvic acid is an important organic acid which is widely used in the chemicals and drugs, as well as agro-chemical industries. It plays a central role in energy metabolism in living organism. Recent evidences suggest that pyruvic acid is the end-product of glucose oxidation and pyruvic acid is finally oxidized to CO_2 and H_2O through citric acid cycle or Krebs cycle. Thiamine acts as a coenzyme of the carboxylase which helps

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in oxidative decarboxylation of pyruvic acid. In the absence of this (Vitamin B_1 or thiamine), pyruvic acid fails to be broken down and hence, accumulates in blood and tissues. This metabolic disorder results in beri-beri and the heart also becomes weak and enlarged which is obviously due to accumulation of pyruvic acid (Li et al., 2001).

Electrochemical methods such as square-wave voltammetry (SWV), stripping voltammetry (SV), differential pulse voltammetry (DPV) and differential pulse polarography (DPP) have been widely applied for the determination of pharmaceuticals. Electrochemical techniques are time saving, cost effective, provide qualitative and quantitative information. Over the last decade, adsorptive stripping voltammetry (AdSV) has been established as a very reliable analytical technique, which is widely recognized as one of the most sensitive methods in electroanalytical chemistry (Shamsipur and Farhadi, 2000; Solangi et al., 2005; Sun et al., 2005; Jain and Rather, 2011; Jain et al., 2007, 2009; Beltagi, 2003; Tamer et al., 2002; Ghoneim and Beltagi, 2003; Ghoneim et al., 2003).

There are few reports in the literature for the determination of pyruvic acid by enzymic fluorescence capillary analysis (Zhao et al., 2008), capillary electrophoresis with amperometric detection (Lu et al., 2007), high performance liquid chromatography (HPLC) (Ewaschuk, 2004) and voltammetry/polarography (Martin et al., 2005; Wang and Diao, 2011). However, reviewing the literature revealed that, up to the present time, there is no adsorptive stripping voltammetric method using hanging mercury drop electrode for the determination of pyruvic acid in solubilized system and biological fluids. The limit of detection is very low as compared to previously reported methods.

Surface active agents play an important role in various fields of pharmaceutical analysis. Surfactants are often used as selective masking agent to improve not only sensitivity but also selectivity of electrochemical methods. Surfactants help in solubilizing the organic compounds and provide specific orientation to the molecule at electrode interface (Fogliatto et al., 2010; Jain and Rather, 2011; Hosseinzadeha et al., 2009; Atta et al., 2007; Gupta et al., 2011; Hoyer and Jensen, 2006).

The purpose of this work is to study the voltammetric reduction behavior of pyruvic acid by employing different voltammetric techniques and to establish the methodology for their trace determination by using square-wave adsorptive stripping voltammetry (SWAdSV) and differential pulse adsorptive stripping voltammetry (DPAdSV) in solubilized system and biological samples.

2. Experimental

2.1. Chemicals and apparatus

All reagents were of AR grade purchased commercially. Pyruvic acid of Sigma, grades was used. Solutions were prepared using double distilled water. Stock standard solution of pyruvic acid was prepared in double distilled water.

The voltammetric studies were carried out in exploratory and determination mode on a software connected Ω Metrohm 797 VA Computrace (ion analyzer). The voltammetric cell consisted of a three electrode assembly and a stirrer with hanging mercury drop electrode as a working electrode, a platinum wire as auxiliary electrode and Ag/AgCl (saturated KCl) electrode as reference electrode. Nitrogen gas was purged through the solution for 5 min. A systronics digital μpH meter model-361 was used for pH measurements.

2.2. Procedure

A 10 ml aliquot of 0.1 M ammonia buffer of desired pH was taken in a clean and dry voltammetric cell and then required standard solution of pyruvic acid was added. The test solution was purged with nitrogen for 5 min initially. The optimized accumulation potential was applied to a HMDE while the solution was stirred for 30 s. Following the pre-concentration period, the cathodic scans were carried out over the range −1.0 to −1.8 V. All measurements were made at room temperature.

2.3. Recovery experiments

To investigate the accuracy and reproducibility of the proposed methods, recovery experiments were carried out using the standard addition method. In order to know whether the excipients show any interference with the analysis, a known amount of pure pyruvic acid was added to the biological sample and this solution was analyzed by the proposed method. The recovery results were determined based on five parallel analyses.

2.4. Analysis of biological samples

Drug-free human blood obtained from healthy volunteers was centrifuged at 5000 rpm for 30 min to separate serum and plasma at room temperature. Separated serum and plasma samples were stored frozen until analysis. Then separated serum and plasma samples were treated with 1.0 mL of acetonitrile as protein denaturing and precipitating agent. After vortexing for 30 s, the serum and plasma samples were centrifuged for 10 min at 5000 rpm in order to eliminate serum and plasma protein residues. An aliquot volume of supernatant of serum, plasma and urine samples was taken carefully for analysis. The concentration of pyruvic acid was varied in the range 0.004–0.036 mM in human serum, plasma and urine samples. The voltammograms were recorded according to the recommended procedure for pyruvic acid. Values of the current (i_p) versus the corresponding concentration were plotted to obtain the calibration graph.

3. Results and discussion

3.1. Electrochemical behavior of pyruvic acid at HMDE

In order to understand the electrochemical process occurring on HMDE, CV, DPAdSV and SWAdSV were carried out. In all electrochemical methods pyruvic acid gave one well-defined reduction peak in the aqueous solution which is attributed to the reduction of unsaturated C═O bond.

3.1.1. Cyclic voltammetric behavior

Pyruvic acid exhibited single well defined cathodic peak in the potential range −1.0 to −1.8 V, at all concentrations. No peak

could be observed in the anodic direction of the reverse scans, suggesting the irreversible nature of the electrode process (Zeng and Huang, 2004). The peak potential shifted toward more negative values of applied potential with increasing scan rate, also confirming the irreversible nature of the reduction process. For a totally irreversible electrode process, the relationship between the peak potential (E_p) and scan rate (υ) is expressed as Brahman et al., 2012; Levent et al., 2009:

$$E_p = (2.303RT\ \alpha_n F)\log(RTK_f/\ \alpha_n F) - (2.303RT\ \alpha_n F)\log\upsilon.$$

A straight line was observed when E_p was plotted against $\log\upsilon$ at a particular concentration at pH 8.2 ± 0.01 and can be expressed as

$$y(E_p) = 0.036\ (\log\upsilon) + 1.5596, r^2 = 0.99$$

From the slope of the straight line ($\Delta E/\Delta\log\upsilon$), the α_n value was calculated by the expression $\Delta E/\Delta\log\upsilon = 30/\alpha_n$. The α_n value was found to be 0.20 and was taken for further calculation for the number of electron transferred. Fractional α_n values further confirmed the irreversible reduction of pyruvic acid.

The effect of scan rate ($\upsilon^{1/2}$) on stripping peak current (i_p) was examined under the above experimental conditions (Fig. 1A). As the sweep rate was increased from 10 to 130 mV s^{-1} at a fixed concentration of pyruvic acid, (i) the peak potential shifted cathodically, (ii) the peak current increased steadily, and (iii) the peak current function, $i_p/AC\upsilon^{1/2}$, exhibited near-constancy.

A straight line is obtained when i_p is plotted against $\upsilon^{1/2}$ (Fig. 1B), which may be expressed by the equation

$$y(i_p) = 14.65\upsilon^{1/2}(\text{mV s}^{-1}) - 36.31, \quad r^2 = 0.974$$

All these facts pointed toward the diffusion-controlled nature of the electrode process. The plot of $\log i_p$ of the peak current in ammonia buffer of pH 8.2 ± 0.01, versus $\log\upsilon$ (Fig. 2) was straight line with slope of 0.755, which is less than the theoretical value of 1.0 that is expected for an ideal reaction of surface species (Levent et al., 2009). For finding the adsorptive character of the drug at HMDE a cyclic voltammogram was recorded after 30 s preconcentration at −0.5 V and second cyclic voltammogram was also recorded at same mercury drop. Furthermore, a substantial decrease in the peak current value in subsequent scans was observed, which reached a steady state, indicating that pyruvic acid also shows adsorptive characteristics at mercury electrode. The lower experimental slope (0.755) than the theoretical one may be attributed to the partial involvement of the diffusive drug molecules in the electrode reaction of the adsorbed ones. The overall electrode process is mainly diffusion-controlled with adsorption of the pyruvic acid at the electrode surface.

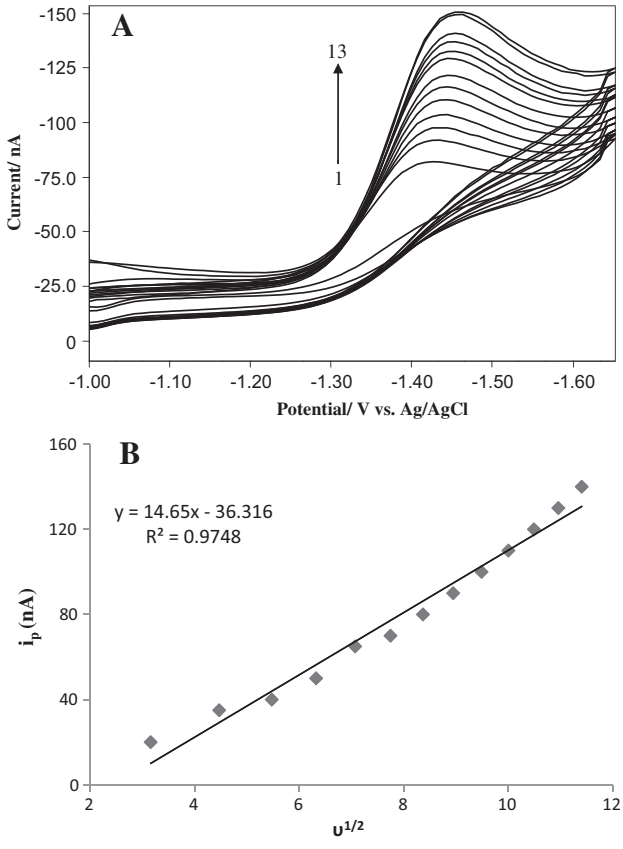


Figure 1 (A) The CVs of 0.004 mM pyruvic acid in the ammonia buffer of pH 8.2 ± 0.01 at HMDE, current range 10 μA. scan rates (mVs^{-1}) (1) 10, (2) 20, (3) 30, (4) 40, (5) 50, (6) 60, (7) 70, (8) 80, (9) 90, (10) 100 (11) 110, (12) 120 and 130. (B) Relationship between peak current (i_p) versus root of scan rate ($\upsilon^{1/2}$).

3.1.2. Differential pulse voltammetric behavior

In differential pulse voltammetry, pyruvic acid shows a single well defined, irreversible, diffusion controlled reduction peak in ammonia buffer solution of pH 8.2 ± 0.01 (Fig. 3), which is attributed to the reduction of the unsaturated C═O bond. When a solution of pyruvic acid (0.004 mM) in 0.1 M ammonia buffer of pH 8.2 ± 0.01, was electrolyzed using HDME as working electrode, it produced well defined reduction peak at −1.35 V.

3.1.3. Effect of CTAB concentration

Squarewave voltammetry shows, cathodic peak current increases steadily in the beginning with an increase in concentration of CTAB and reaches a maximum at 4 mM CTAB and after that decreases continuously (Fig. 4A). It may be interpreted that at 4 mM CTAB the adsorption behavior changes

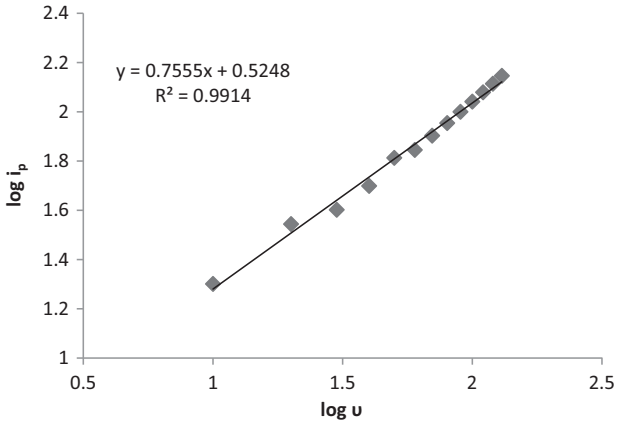


Figure 2 Relationship between log of peak current ($\log i_p$) versus log of scan rate (υ).

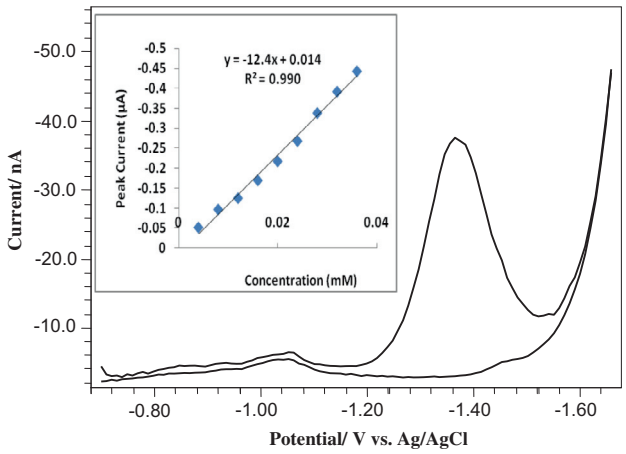


Figure 3 Differential pulse adsorptive stripping voltammogram of pyruvic acid (0.004 mM) in 0.1 M ammonia buffer and 4 mM CTAB (pH 8.2 ± 0.01) at HMDE, pulse amplitude −50 mV, scan rate 30 mVs^{-1}, current range 10 μA, accumulation time 30 s.

from monomer adsorption to monolayer adsorption with an increase in concentration of CTAB at the electrode surface. However peak current decreases with a further increase in CTAB concentration, it may be due to the inhibition of electron transfer by aggregates of micelles. Another reason for decrease in peak current is the increase of hydrophobicity of CTAB micelles that might decrease the electron transfer rate constant (Jain and Rather, 2011; Brahman et al., 2012; Tan et al., 1997) and result in the decrease of peak current at high CTAB concentration. Therefore, 4 mM concentration of CTAB was chosen as optimum one.

3.2. Optimization conditions for the determination of pyruvic acid

Variation of the stripping voltammetric peak current of pyruvic acid at HMDE was investigated using square-wave and differential pulse modes. Both the techniques gave comparable results but square-wave adsorptive stripping voltammetry has been chosen for optimizing the operational parameters. The SWV technique is more sensitive than DPV and CV, because in SWV both forward and reverse current are measured while only forward current is measured in DPV and also the scan rate determined by the SW frequency (20–90 Hz) is much faster than DPV. The peak current obtained in SWV is about four times higher than differential pulse response. The important instrumental variables in SWV are accumulation time, accumulation potential; pulse amplitude, scan increment and frequency were examined.

3.2.1. Effect of accumulation time and potential

Variation of accumulation time for 0.004 mM pyruvic acid solution was studied over 0–60 s. A remarkable enhancement for the SWAdSV peak current up to 30 s accumulation time was observed and then it became virtually curved due to the saturation of the surface of the working electrode. For further SWAdSV quantitative studies for pyruvic acid, an accumulation time of 30 s was selected as optimal value since it provided relatively high peak current with adequate practical time. In addition, when the influence of pre-concentration potential over the range of −1.2 V to +0.2 V at 30 s accumulation time, the peak current increased steadily over the positive direction till it reached its maximum value at −0.5 V where it decreased sharply thereafter. Hence, for optimal analytical sensitivity, this experimental parameter was maintained at −0.5 V.

3.2.2. Influence of frequency

The SWAdSV peak current is linearly dependent on the frequency 20–90 Hz, while a well defined peak was observed at 20 Hz. A linear relationship was observed between the stripping peak current and frequency. Although the response of pyruvic acid is increased with frequency, above 70 Hz the peak current was obscured by a large residual current. Thus the best peak was recorded using 20 Hz frequency.

3.2.3. Influence of scan increment and pulse amplitude

The effect of scan increment on adsorptive cathodic peak current of the pyruvic acid in ammonia buffer at pH 8.2 ± 0.01 revealed that the peak current increases upon the increase of scan increment (1–20 mV). The best peak resolution was recorded using 5 mV scan increment. Thus scan increment of 5 mV was used in the present study. At pulse amplitude of 50 mV, the peak current was found to be much more sharp

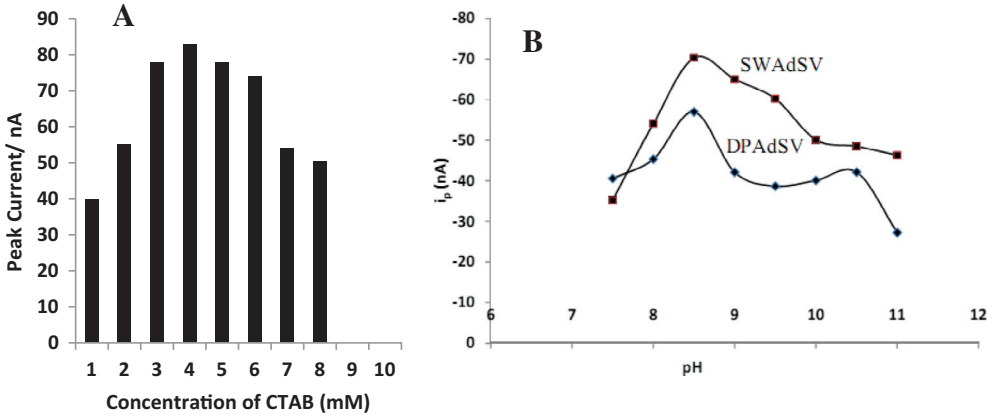
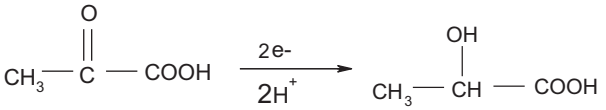


Figure 4 (A) Plot of square wave adsorptive peak current vs. concentration of CTAB in ammonia buffer, pH 8.2 ± 0.01. (B) Influence of pH on the peak current response (using DPAdSV and SWAdSV) for 0.004 mM pyruvic acid in ammonia buffer (pH 7.5–11) and 4 mM CTAB after 30 s pre-concentration time; frequency (f) = 20 Hz, and pulse amplitude = 50 mV at Eacc = − 0.5 V.



Scheme 1 Probable reduction of pyruvic acid.

and defined. Several instrumental parameters, those directly affect voltammetric response, were also optimized for e.g., mercury drop size, stirring rate and the rest period. The stripping was not significantly affected when varying the rest period, since it was observed that 10 s was sufficient for the formation of a uniform concentration of the reactant onto the mercury drop.

3.2.4. Effect of pH

The shape and characteristics of all voltammograms were strongly dependent on various electrolyte and pH of the medium. Britton–Robinson, acetate, borate, citrate, phosphate, Tris and ammonia buffer were used in the present study and the best results were obtained in ammonia buffer (0.1 M). Stripping peak potential shifted toward more negative potential with an increase in pH, indicating involvement of hydrogen ions in the electrode process. Variation of stripping peak potential of pyruvic acid as a function of pH (7.5–11) employing different voltammetric modes can be expressed by the following equations:

$$\mathrm{DPAdSV; pH7.5-11.0:}\ E_p(V) = -0.0231 - 1.124\mathrm{pH}:\ r^2 = 0.9829$$

$$\mathrm{SWAdSV; pH7.5-11.0:}\ E_p(V) = -0.021 - 1.102\mathrm{pH}:\ r^2 = 0.9912$$

Studies for the dependence of stripping peak on pH for DPAdSV and SWAdSV (Fig. 4B) were carried out to determine whether the electro-active species participate in equilibria involving protons directly and to obtain the pH range for maximum signal. Sharp response and better peak shape with maximum current were observed at pH 8.2 ± 0.01. So this pH value was chosen as the working pH for further studies.

3.3. Measurement of the number of electrons and protons transferred

The plot of peak potential versus pH gave a slope of 56 mV pH^{-1} which is close to the expected value of 59 mV pH^{-1}. With this, we could propose that the number of electrons and protons participating in the electrode process are equal i.e. during the reaction not only electrons but also protons are released from the molecule. The electron transfer coefficient 'α' is calculated from the difference between peak potential (E_p) and half wave potential ($E_{p/2}$) according to the equation given below for the electrode process (Tan et al., 1997; Dar et al., 2011, 2012a,b).

$$\Delta E = E_p - E_{p/2} = (47.7/\alpha)\mathrm{mV\ (for\ electrode\ process\ at\ 298K)}$$

The value of α is calculated to be 0.53.

According to the Laviron equation (Hosseinzadeha et al., 2009) for the irreversible reduction wave:

$$W_{1/2} = 2.44RT/(\alpha_n F) = 62.5/(\alpha_n)(298\mathrm{K})$$

where $W_{1/2}$ is the half width of the peak. From the peak, $W_{1/2}$ = 68.5 mV. The same process was repeated three times and gave n = 1.859, 1.71, 2.02.

Thus, we concluded that pyruvic acid in 0.1 M ammonia buffer undergoes two electron irreversible reduction reactions. The only one expected reduction product of pyruvic acid on electrode surface is lactic acid (Scheme 1).

3.4. Determination of pyruvic acid

The applicability of the proposed method as an analytical method for the determination of pyruvic acid was examined by measuring the stripping peak current as a function of concentration of the bulk drug at least three times under the optimized operational parameters. A calibration graph for the pyruvic acid was recorded to estimate the analytical characteristics of the developed method when the most ideal and suitable chemical conditions and instrumental parameters for the voltammetric determination were established. Two calibration graphs were constructed. The dependency that existed between stripping peak current and concentration of the pyruvic acid was linear with a range of 0.004–0.036 mM for SWAdSV and DPAdSV (Fig. 5). The calibration graph was represented by the equation:

$$\mathrm{SWAdSV:}\ i_p(\mu A) = -29.83x + 0.010\ r^2 = 0.995$$

$$\mathrm{DPAdSV:}\ i_p(\mu A) = 12.4x + 0.014\ r^2 = 0.999$$

The regression plots showed that there was a linear dependence of the current intensity on the concentration in both the DPAdSV and SWAdSV modes over the range as given in Table 1. The table also shows the LODs and results of the statistical analysis of the experimental data such as slopes, intercepts, and correlation coefficients obtained by linear least squares treatment of the results. The good linearity of the calibration graphs and the negligible scatter of the experimental points are clearly evident by the values of the correlation coefficients and standard deviations.

3.5. Analysis of pyruvic acid in biological samples

In order to evaluate the applicability of the method to biological samples, pyruvic acid was determined in serum, plasma and urine samples under the same conditions as employed for the pure pyruvic acid by using the standard addition method. Table 2 shows the results obtained for pyruvic acid in the corresponding biological samples together with DPAdSV and SWAdSV analysis. The content of pyruvic acid in serum, plasma and urine is calculated and it is 0.018, 0.016 and 0.021 mg/ml which is in accordance with that reported in the literature (Zhao et al., 2008; Lu et al., 2007). The recoveries are in good agreement with the RSD values that are less than 1%. Thus, the precision is very satisfactory for the analysis of biological samples. These results indicate that the content of pyruvic acid in the biological samples can be safely determined by using this method without interference from other substances in the blood samples. The recovery studies of standard additions to biological samples were carried out in order to provide further

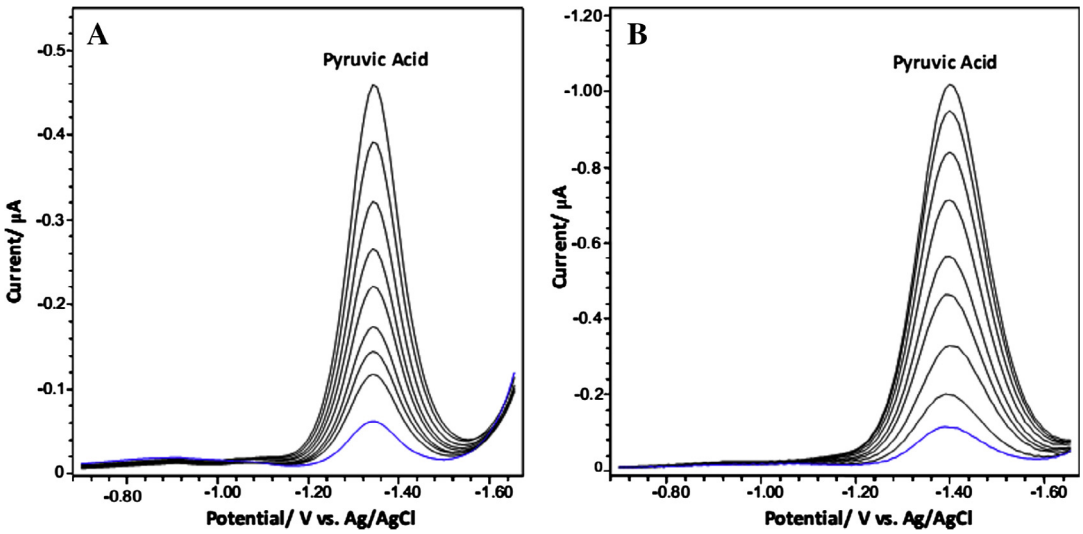


Figure 5 Voltammograms of pyruvic acid using (A) DPAdSV and (B) SWAdSV at different concentrations including: (1) 0.004 (2) 0.008 (3) 0.012 (4) 0.016 (5) 0.02 (6) 0.024 (7) 0.028 (8) 0.032 (9) and 0.036 mM, pH 8.2, accumulation potential −0.5 V, accumulation time 30 s.

Table 1 Regression data of the calibration lines for quantitative determination of pyruvic acid using DPAdSV and SWAdSV.

Parameters	DPAdSV	SWAdSV
Measured Potential (V)	−1.35	−1.38
Linearity range (mM)	0.004–0.036	0.004–0.036
Slope (µA/mM)	−12.4	−29.83
Intercept (µA)	0.014	0.010
Correlation coefficient (r^2)	0.995	0.999
Limit of detection (LOD) (mM)	6.12×10^{-6}	1.12×10^{-7}
Limit of quantification (LOQ) (mM)	2.0×10^{-5}	4.4×10^{-6}
Repeatability of peak current (RSD%)	1.4	0.9
Repeatability of peak potential (RSD%)	0.9	1.0
Reproducibility of peak current (RSD%)	1.11	0.8
Reproducibility of peak potential (RSD%)	1.3	1.0

evidence of validity of the methods. It can be seen from this table that the mean recoveries and RSD values for DPAdSV and SWAdSV are in the range of 99.45–101.70%, which is good evidence of validity of method. As can be seen in Table 2, both DPAdSV and SWAdSV methods were applied to biological samples after a simple dilution step with direct measurements.

3.6. Analytical performance of the developed procedure

Once the most ideal and suitable chemical conditions and instrumental parameters for the adsorptive stripping voltammetric determination were established, a calibration plot for the analyzed drug was recorded to estimate the analytical characteristics of the developed method.

Table 2 Analytical results for pyruvic acid in serum, plasma and urine.

Technique	Medium	Added (µg mL^{-1})[a]	Expected (µg mL^{-1})[a]	Found (µg mL^{-1})[a]	% Recovery	RSD%
SWAdSV	Serum	Nil	–	5.431	–	0.87
		10	15.431	15.381	99.67	0.95
		20	25.431	25.393	99.85	0.67
	Plasma	Nil	–	5.102	–	0.84
		10	15.102	14.942	98.94	0.79
		20	25.102	25.012	99.64	0.93
	Urine	Nil	–	6.420	–	0.94
		10	16.420	16.452	100.2	1.02
		20	26.420	26.414	99.97	0.86
DPAdSV	Serum	Nil	–	5.031	–	1.20
		10	15.031	14.748	98.11	1.03
		20	25.031	24.643	98.44	0.99
	Plasma	Nil	–	4.802	–	1.31
		10	14.802	14.671	99.11	0.84
		20	24.802	24.30	97.97	0.92
	Urine	Nil	–	5.983	–	0.87
		10	15.983	15.752	98.55	1.11
		20	25.983	25.717	98.97	1.0

[a] Average of five replicate measurements.

3.6.1. Linearity

In order to determine the effect of concentration of pyruvic acid on stripping peak current, voltammograms of pyruvic acid were recorded at HMDE. Under the optimum conditions, a very good linear correlation was obtained between the monitored voltammetric peak current and pyruvic acid concentration in the range of 0.004–0.036 mM. Least-square treatment of the calibration graph yielded the following regression equations:

$$\text{SWAdSV}: \ i_p(\mu A) = -29.83x + 0.010\, r^2 = 0.995$$

$$\text{DPAdSV}: \ i_p(\mu A) = 12.4x + 0.014\, r^2 = 0.999$$

where i_p is the peak current, x is the analyzed drug concentration and r^2 is the correlation coefficient.

3.6.2. Detection and quantification limits

Detection limit is calculated by equation LOD = 3s/m, where s is standard deviation of intercept and m is slope of the regression line. The calculated LOD value of pyruvic acid is 6.12×10^{-6} and 1.12×10^{-7} mM for DPAdSV and SWAdSV, respectively. The quantification limit (LOQ) is examined by the equation LOQ = 10s/m. The calculated LOQ value is 2.0×10^{-5} and 4.4×10^{-6} mM for DPAdSV and SWAdSV, respectively. Both LOD and LOQ values confirmed the sensitivity of the proposed methods.

3.6.3. Accuracy, precision and stability

The accuracy of the proposed method was checked by calculating the recovery of known amount of pyruvic acid (0.004 mM) added to ammonia buffer solution and analyzed via the optimized stripping voltammetric procedure. The value of the mean recovery obtained by the standard addition method was 100.9% with standard deviation of 1.2% (the analytical measurements repeated five times). The analytical precision of the developed method was verified from the reproducibility of 10 determinations of 0.004 mM pyruvic acid and the estimated relative standard deviation (RSD%) was 1.07%. Finally, when the SWAdSV signal of 0.004 mM pyruvic acid solution was monitored every fifteen minutes, it was found to be nearly stable for a period of at least 2 h.

3.6.4. Robustness

The robustness was examined by evaluating the influence of small variations of some of the most important procedure variables, including preconcentration potential, preconcentration time, and pH. The obtained results provided an indication of the reliability of the proposed procedure for the assay of pyruvic acid; hence, it can be considered as robust. The obtained mean percentage recoveries based on an average of five replicate measurements were not significantly affected within the studied range of variations of some operational parameters, and consequently the proposed procedure can be considered as robust.

3.6.5. Ruggedness

The ruggedness of the measurements is defined as the degree of reproducibility of results obtained by analysis of same sample under a variety of normal test conditions such as different laboratories and different lot of reagents, under the same operational conditions at different elapsed time by two different analysts. The methods were found to be rugged with the results of variation coefficients 0.85% and 0.91% for SWAdSV, 1.3% and 0.94% for DPAdSV methods for first and second analysts, respectively. The results show no statistical differences between different analysts.

3.6.6. Specificity/selectivity

Specificity is the ability of the method to measure the analyte response in the presence of all of the potential impurities. The selectivity of the optimized procedure for determination of pyruvic acid was examined in the presence of foreign species such as Ca^{2+}, Mg^{2+}, Zn^{2+}, Al^{3+}, Cu^{2+}, glucose, valine, phenylalanine and lycine. Samples containing 0.004 mM bulk pyruvic acid and different concentrations of the excipient under evaluation were analyzed by means of the proposed method. The obtained mean percentage recoveries and RSD% values based on an average of five replicate measurements, 99.90 ± 0.54–100.10 ± 1.20 for SWAdSV and 99.21 ± 1.7–100.06 ± 0.90 for DPAdSV, showed no significant interference from excipients. Thus, the proposed procedure can be considered to be specific.

3.6.7. Comparison of the sensitivity of the proposed method with previously reported methods

Table 3 compares the detection limit of the proposed method with the other reported methods. It is obvious that the sensitivity of the proposed method is superior to all previously reported methods. The data in the table reveal that the detection limit of the method is lower than all previously reported methods.

4. Conclusion

Sensitive adsorptive stripping voltammetric methods have been developed for the determination of pyruvic acid in solubilized system at HMDE. The proposed adsorptive stripping voltammetric procedure can be used successfully to determine pyruvic acid in human plasma, serum and urine. The electrochemical reduction of pyruvic acid under the conditions described in this work is an irreversible process controlled by adsorption. The present method is found to be practically

Table 3 Comparison of various existing assays with square wave adsorptive stripping voltammetric analysis of pyruvic acid.

Method	Detection limit (mM)	Refs. no.
Enzymic fluorescence capillary analysis	1.2×10^{-2}	Zhao et al. (2008)
Capillary electrophoresis	3.88×10^{-4}	Lu et al. (2007)
Voltammetry/polarography	5.5×10^{-4}	Wang and Diao (2011)
Square wave adsorptive stripping voltammetry	1.12×10^{-7}	Present work

rapid, convenient, accurate, low cost and precise. As applied to serum, plasma and urine samples, these methods have the advantage that no prior extraction procedure is required prior to the analysis. The developed method with the detection limit of 1.12×10^{-7} mM is more sensitive than already reported analytical methods. The method could possibly be adopted for the pharmacokinetic studies as well as for quality control laboratories.

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